PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 39/395 — A1

(11) International Publication Number: WO 99/37329

(43) International Publication Date: 29 July 1999 (29.07.99)

(21) International Application Number: PCT/SE99/00049

(22) International Filing Date: 15 January 1999 (15.01.99)

(30) Priority Data:

9800170-4	22 January 1998 (22.01.98)	SE
9800756-9	9 March 1998 (09.03.98)	SE

(71) Applicant (*for all designated States except US*): ASTRA AKTIEBOLAG [SE/SE]; S-151 85 Södertälje (SE).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): FLINK, Ola [SE/SE]; Astra Arcus AB, S-151 85 Södertälje (SE). PETRÉN, Sven [SE/SE]; Astra Arcus AB, S-151 85 Södertälje (SE).

(74) Agent: ASTRA AKTIEBOLAG; Intellectual Property, Patents, S-151 85 Södertälje (SE).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

(54) Title: PHARMACEUTICAL FORMULATION COMPRISING AN ANTIBODY AND A CITRATE BUFFER

(57) Abstract

The present invention relates to an isotonic pharmaceutical formulation comprising an antibody and a buffer, wherein the antibody is present at 0.5 mg/ml to 10 mg/ml, the buffer is a citrate buffer present at 5 mmol/l to 20 mmol/l and the pH of the formulation is 5.3 to 7.2. The invention also relates to a method for the storage of antibodies by use of such formulations and to the use of such formulations in therapy.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

PHARMACEUTICAL FORMULATION COMPRISING AN ANTIBODY AND A CITRATE BUFFER

The invention relates generally to antibody formulations and particularly to stabilised antibody formulations for storage and for therapeutic administration.

Purified proteins, particularly those produced using recombinant DNA technology are now well established as pharmaceutical agents. Such proteins do however present a range of problems associated with their stable formulation. Many protein preparations are particularly unstable in dilute solutions and must be formulated in such a way as to prevent significant levels of denaturation, agglomeration or degradation. These problems are particularly acute in the formulation of large proteins such as immunoglobulins. Immunoglobulins or antibodies are known to be prone to form aggregates and particulates in solution and this has long provided special problems in generating suitable formulations for the storage and administration of therapeutic antibodies. Existing antibody formulations frequently require to be filtered before injection to remove aggregates or particulate matter which is inconvenient and tends to reduce the accuracy of the injected dose.

Various attempts have been made to overcome the problems of antibody formulation:

EP 0 073 371 describes intravenously administrable immunoglobulin compositions which have their pH adjusted to 3.5 to 5.0 as proteins are known to be more stable at low pH. Such low pHs however tend to result in undesirable reactions at the site of injection .

US 4650772 describes a method for stabilising thermally unstable monoclonal antibodies which requires the presence of 0.25% to 5% hydrolysed ovalbumin. The use of ovalbumin in pharmaceutical formulations results in the induction of an allergic response which prevents its effective use for repeated administrations.

WO 90/11091 describes the use of maltose and buffers in a lyophilised formulation of monoclonal antibodies. Lyophilisation is however an expensive process and the need to

resuspend the formulation prior to administration adds to the complexity of the treatment regimen. It was suggested that citrate buffer may be used to buffer the pH at between 3.0 and 6.0.

The present invention provides a more simple antibody formulation than those presently known, providing a formulation which is both suitable for administration and has improved storage properties. Existing antibody formulations require the use both of a stabiliser and of a buffer. However antibodies in formulations of the present invention are stabilised only by citrate buffer in a saline solution at a physiologically preferable pH.

There is therefore provided according to the present invention, an isotonic pharmaceutical formulation comprising an antibody and a buffer, wherein the antibody is present at 0.5mg/ml to 10mg/ml, the buffer is a citrate buffer present at 5mmol/l to 20mmol/l and the pH of the formulation is 5.3 to 7.2.

A citrate buffer for use in the present invention may be generated by dissolution of free citric acid or preferably a pharmaceutically acceptable salt of citrate, preferably a sodium salt.

A formulation of the present invention may be generated by solubilising the relevant antibody, preferably in saline and adding an amount of citrate buffer necessary to obtain a pH of the solution in the range 5.3 to 7.2. The citrate buffer is preferably present at 5mmol/l to 20mmol/l.

A formulation of the present invention may additionally contain other substances desirable for therapeutic efficacy of the antibody e.g. chelators, or other therapeutic compounds desirable to be coformulated with the antibody but it is preferably substantially free of any additional compound known for use in antibody stabilising e.g. Tween, mannitol or maltose. By 'substantially free' it is meant that such additional compounds known for use in antibody stabilising formulations may not be present in formulations of the present

invention in an amount capable alone or in combination with one or more other stabilisers, of having a stabilising effect upon an antibody in a formulation.

In preferred embodiments of the present invention citrate buffer is present in the formulation at 7.5mmol/l to 15mmol/l and most preferably at 10mmol/l. Any pharmaceutically acceptable citrate buffer may be used in the present invention but the citrate buffer is preferably sodium citrate. It is more preferable that sodium citrate dihydrate is used and most preferable that the citrate buffer be generated from a mixture of sodium citrate dihydrate and citric acid monohydrate. In the preferred embodiments of the present invention, the formulation contains about 2.4 mg/ml sodium citrate dihydrate and about 0.387 mg/ml citric acid monohydrate.

The present invention is suitable for the formulation of any antibody or antibody fragment. Any reference to an antibody herein will be taken to include a fragment of such antibody. The antibody for use in a formulation of the present invention may be natural or recombinant and may be generated according to any known technique. Natural antibodies may be those isolated either by purification from body fluids or from cell lines and may be polyclonal or monoclonal antibodies. Particularly preferred antibodies for use in formulations of the present invention are recombinant antibodies produced from engineered cell lines. Such cell lines will have been engineered to express the relevant antibody gene. The antibody gene may either be a human gene or a gene from another species which has been humanised by modification of the native sequence to prevent rejection when administered to a human, e.g. a humanised recombinant antibody. The antibody is preferably an antibody directed against the human T cell surface receptor TCR Vβ 5.2/5.3 (the method for constructing such an antibody is described in WO 95/16038 and the description of such methods is hereby incorporated by reference) , and is more preferably an IgG, IgG1 or IgG/κ. The antibody is most preferably the antibody produced by the cell line deposited on June 22, 1995 under the Budapest treaty as ATCC (CRL 11949) [herein this antibody is referred to as 'TM27'] or is one comprising the following TM27 Vκ sequence:

1 DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQTPGKAPKLLIYY 50

51 TSSLHSGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQYSKLPRTFGQ 100

101 GTKLQIT 107,

and further comprises an amino acid sequence selected from the group consisting of the TM27 VH amino acid sequence:

1 QVQLQESGPGLVRPSQTLSLTCTVSGFSLTAYGVNWVRQPPGRGLEWLGM 50

51 IWGDGNTDYNSALKSRVTMLKDTSKNQFSLRLSSVTAADTAVYYCARDRV 100

101 TATLYAMDYWGQGSLVTVSS 120,

the TM27 VH sequence wherein amino acid residue 48 is replaced with isoleucine (I),

the TM27 VH sequence wherein amino acid residues 78 and 79 are valine (V) and phenylalanine (F),

theTM27 VH sequence above wherein amino acid residues 67 to 70 VTML are replaced with LSIS respectively and amino acid 73 is an aspargine (N),

the TM27 VH sequence wherein amino acid residue 92 is an arginine (R).

The present invention also provides for the use of formulations of the present invention in medical therapy and particularly for the treatment of autoimmune disease and further particularly in the therapy of multiple sclerosis.

In preferred embodiments of the present invention the formulation has a pH in the range 5.5 to 6.5 and is most preferably pH 5.5. The pH may be altered using any pharmaceutically acceptable acid or alkali.

The formulation of the present invention may be prepared under aseptic conditions, leading to a sterile formulation.

The invention will now be illustrated by reference to the following examples which are in no way intended to be limiting of the scope of the invention described herein.

EXAMPLES

The antibody assayed in the following examples is TM27 which is a humanised antibody (IgG1) produced in Chinese hamster ovary (CHO) cells by recombinant technology.

The bulk batches of TM27 used were purified by protein-A affinity chromatography. The TM27 preparations were prepared from two different TM27 bulk solutions. One was 10.8 mg TM27 /ml in 10 mmol/l citrate buffer pH 5.5 and the other was 11.0 mg TM27 /ml in 10 mmol/l phosphate buffer pH 6.5. The bulk solutions had sodium chloride added to make them isotonic.

The TM27 preparations used in this study were prepared under aseptic conditions. The batches were protected from air with nitrogen during the manufacturing and filling processes. Eight batches with different compositions were prepared using the buffers described.

For manufacturing purposes, TM27 bulk solution was diluted with the appropriate buffer to a concentration of 1 mg/ml TM27 by gentle mixing, while avoiding foaming. The manufactured solutions were filtered through a sterile 0.22 µm MILLEX-GV filter directly into 10 ml sterile glass vials. Filling was performed from the bottom of the vials under nitrogen protection.

The solutions were filled into 10 ml injection vials of neutral Type I glass (Ph Eur), 1 ml/vial. Bromobutyl rubber stoppers (FM 257) were used and the vials were sealed with aluminium capsules.

The batches were stored under the following conditions: +5 °C/ambient humidity and +25 °C/30 % relative humidity. All the vials were stored upright. All examples contain antibody at 1 mg/ml.

Example 1: 10mmol/l citrate, pH 5.5	amount (mg)
Citric acid monohydrate for parenteral use	0.387
Sodium citrate dihydrate for parenteral use	2.400
Sodium chloride for aseptic preparation	8.4
Water for injection	to 1 ml

Example 2: 5 mmol/l citrate ,pH 5.5	
Citric acid monohydrate for parenteral use	0.194
Sodium citrate dihydrate for parenteral use	1.200
Sodium chloride for aseptic preparation	8.69
Water for injection	to 1 ml

Example 3: 8 mmol/l citrate, pH 5.5	
Citric acid monohydrate for parenteral use	0.310
Sodium citrate dihydrate for parenteral use	1.920
Sodium chloride for aseptic preparation	8.52
Water for injection	to 1 ml

Example 4: 12 mmol/l citrate, pH 5.5	
Citric acid monohydrate for parenteral use	0.464
Sodium citrate dihydrate for parenteral use	2.88
Sodium chloride for aseptic preparation	8.28
Water for injection	to 1 ml

Example 5: 15 mmol/l citrate, pH 5.5

Citric acid monohydrate for parenteral use	0.581
Sodium citrate dihydrate for parenteral use	3.600
Sodium chloride for aseptic preparation	8.11
Water for injection	to 1 ml

Example 6: 18 mmol/l citrate, pH 5.5

Citric acid monohydrate for parenteral use	0.697
Sodium citrate dihydrate for parenteral use	4.320
Sodium chloride for aseptic preparation	7.93
Water for injection	to 1 ml

Example 7: 20 mmol/l citrate, pH 5.5

Citric acid monohydrate for parenteral use	0.774
Sodium citrate dihydrate for parenteral use	4.800
Sodium chloride for aseptic preparation	7.82
Water for injection	to 1 ml

Example8 (pH 5.5)

Citric acid monohydrate for parenteral use	0.387 mg
Sodium citrate dihydrate for parenteral use	2.400 mg
Sodium chloride for aseptic preparation	8.4 mg

Polysorbat 80 (Tween 80)	0.2 mg
Water for injection	to 1 ml

The formulations were studied over a 24 month period.

The results clearly showed that storage temperature, buffer composition and pH influence the stability of TM27. The best stability is achieved at low storage temperature (+5 °C). The formulations exemplified in examples 1-7 were stable for 24 months whereas the formulation in example 8 was stable for 12 months.

CLAIMS.

1. An isotonic pharmaceutical formulation comprising an IgG antibody and a buffer, wherein the antibody is present at from 0.5 mg/ml to 10 mg/ml, the buffer is a citrate buffer present at 5mmol/l to 20mmol/l and the pH of the formulation is 5.3 to 7.2.

2. A formulation according to claim 1, wherein the pH is 5.5 to 6.5.

3. A formulation according to claim 1, wherein the pH is 5.5.

4. A formulation according to any one of claims 1 to 3, wherein the citrate is present at 7.5mmol/l to 15mmol/l .

5. A formulation according to any one of claims 1 to 4, wherein the citrate is present at 10mmol/l.

6. A formulation according to any one of claims 1 to 5, wherein the antibody is an IgG1.

7. A formulation according to claim 6, wherein the antibody is an IgG1/κ.

8. A formulation according to any one of the preceding claims, wherein the antibody is a recombinant antibody.

9. A formulation according to claim 8, wherein the antibody is a humanised recombinant antibody.

10. A formulation according to claim 9, wherein the antibody is directed against the human T cell surface receptor TCR Vβ 5.2/5.3.

11. A formulation according to claim 10, wherein the antibody is produced by the cell line with the deposition number CRL 11949.

12. A formulation according to claim 11, wherein the antibody comprises the TM27 Vκ amino acid sequence:

1 DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQTPGKAPKLLIYY 50

51 TSSLHSGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQYSKLPRTFGQ 100

101 GTKLQIT 107,

and further comprises an amino acid sequence selected from the group consisting of the TM27 VH amino acid sequence:

1 QVQLQESGPGLVRPSQTLSLTCTVSGFSLTAYGVNWVRQPPGRGLEWLGM 50

51 IWGDGNTDYNSALKSRVTMLKDTSKNQFSLRLSSVTAADTAVYYCARDRV 100

101 TATLYAMDYWGQGSLVTVSS 120,

the TM27 VH sequence wherein amino acid residue 48 is replaced with isoleucine (I),

the TM27 VH sequence wherein amino acid residues 78 and 79 are valine (V) and phenylalanine (F),

theTM27 VH sequence above wherein amino acid residues 67 to 70 VTML are replaced with LSIS respectively and amino acid 73 is an aspargine (N),

the TM27 VH sequence wherein amino acid residue 92 is an arginine (R).

13. An isotonic pharmaceutical formulation comprising the antibody TM27 and a buffer, wherein the antibody is present at 0.5 mg/ml to 10 mg/ml, the buffer is a citrate buffer present at 5mmol/l to 20mmol/l and the pH of the formulation is 5.3 to 7.2.

14. A formulation according to any one of claims 1 to 13, which is a sterile formulation.

15. A process for the preparation of an isotonic pharmaceutical formulation according to any one of claims 1 to 14, comprising incorporation of the antibody in an isotonic medium wherein the antibody is present at from 0.5 mg/ml to 10 mg/ml, the buffer is a citrate buffer present at 5mmol/l to 20mmol/l and the pH of the formulation is 5.3 to 7.2.

16. A formulation according to any one of the previous claims, for use in medical therapy.

17. A formulation according to any one of claims 1 to 14, for use in the therapy of an autoimmune disease.

18. A formulation according to any one of claims 1 to 14, for use in the therapy of multiple sclerosis.

19. Use of a formulation according to any one of claims 1 to 14, in the preparation of a medicament for use in the therapy of an autoimmune disease.

20. Use of a formulation according to any one of claims 1 to 14, in the preparation of a medicament for use in the therapy of multiple sclerosis.

21. A method for improving the storage of an antibody comprising formulating the antibody in a formulation as claimed in any one of claims 1 to 14.

22. A method according to claim 21, wherein the formulation is stored at a temperature between 4°C and 10°C.

23. A method according to claim 22, wherein the temperature is 5°C.

SEQUENCE LISTING

<110> Lundquist, Tomas

<120> Sequence Listing

<130> 3526/00000

<140> 00/000,000

<141> 1998-11-25

<150> 00/000,000

<151> 1998-11-24

<160> 6

<170> PatentIn Ver. 2.0

<210> 1

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:humanized monoclonal antibody

<400> 1

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly
1				5				10						15	

Asp	Arg	Val	Thr	Ile	Thr	Cys	Ser	Ala	Ser	Gln	Gly	Ile	Ser	Asn	Tyr
			20					25					30		

Leu	Asn	Trp	Tyr	Gln	Gln	Thr	Pro	Gly	Lys	Ala	Pro	Glu	Leu	Leu	Ile
		35					40					45			

Tyr	Tyr	Thr	Ser	Ser	Leu	His	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				

Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Thr	Phe	Thr	Ile	Ser	Ser	Leu	Gln	Pro
65				70				75						80	

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Lys Leu Pro Arg

85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr

100 105

<210> 2

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:humanized monoclonal antibody

<400> 2

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln

1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr

20 25 30

Gly Val Asn Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Leu

35 40 45

Gly Met Ile Trp Gly Asp Gly Asn Thr Asp Tyr Asn Ser Ala Leu Lys

50 55 60

Ser Arg Val Thr Met Leu Lys Asp Thr Ser Lys Asn Gln Phe Ser Leu

65 70 75 80

Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala

85 90 95

Arg Asp Arg Val Thr Ala Thr Leu Tyr Ala Met Asp Tyr Trp Gly Gln

100 105 110

Gly Ser Leu Val Thr Val Ser Ser

115 120

<210> 3

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:humanized monoclonal antibody

<400> 3

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr
20 25 30

Gly Val Asn Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Met Ile Trp Gly Asp Gly Asn Thr Asp Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Val Thr Met Leu Lys Asp Thr Ser Lys Asn Gln Phe Ser Leu

65 70 75 80

Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala

85 90 95

Arg Asp Arg Val Thr Ala Thr Leu Tyr Ala Met Asp Tyr Trp Gly Gln

100 105 110

Gly Ser Leu Val Thr Val Ser Ser

115 120

<210> 4

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:humanized

monoclonal antibody

<400> 4

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr
20 25 30

Gly Val Asn Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Leu
35 40 45

Gly Met Ile Trp Gly Asp Gly Asn Thr Asp Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Val Thr Met Leu Lys Asp Thr Ser Lys Asn Gln Val Phe Leu
65 70 75 80

Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asp Arg Val Thr Ala Thr Leu Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Ser Leu Val Thr Val Ser Ser

115 120

<210> 5

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:humanized monoclonal antibody

<400> 5

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln

1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr

20 25 30

Gly Val Asn Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Leu

35 40 45

Gly Met Ile Trp Gly Asp Gly Asn Thr Asp Tyr Asn Ser Ala Leu Lys

50 55 60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Asn Gln Phe Ser Leu

65 70 75 80

Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala

85 90 95

Arg Asp Arg Val Thr Ala Thr Leu Tyr Ala Met Asp Tyr Trp Gly Gln

100 105 110

Gly Ser Leu Val Thr Val Ser Ser

115 120

<210> 6

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:humanized monoclonal antibody

<400> 6

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr
20 25 30

Gly Val Asn Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Leu
35 40 45

Gly Met Ile Trp Gly Asp Gly Asn Thr Asp Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Val Thr Met Leu Lys Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Arg Tyr Tyr Cys Ala

85 90 95

Arg Asp Arg Val Thr Ala Thr Leu Tyr Ala Met Asp Tyr Trp Gly Gln

100 105 110

Gly Ser Leu Val Thr Val Ser Ser

115 120

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00049

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 39/395
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9311794 A1 (XOMA CORPORATION), 24 June 1993 (24.06.93), See especially page 34, lines 3-15	1,2,4,5, 14-21
Y	--	1-18
Y	WO 9516038 A2 (T CELL SCIENCES, INC.), 15 June 1995 (15.06.95), See especially abstract, claim 2 and page 60, lines 1-24	6-10,12-13, 17-20
	--	
Y	WO 9011091 A1 (CENTOCOR, INC.), 4 October 1990 (04.10.90), See abstract and claims 1-3	1-18
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 March 1999	20 -04- 1999
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00049

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0531539 A1 (MITSUI TOATSU CHEMICALS, INC.), 17 March 1993 (17.03.93), See claims 2 and 3 --	1,2
A	WO 8911298 A1 (CENTOCOR, INC.), 30 November 1989 (30.11.89) --	

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/03/99

International application No.
PCT/SE 99/00049

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9311794 A1	24/06/93	CA 2103887 A	14/06/93
		EP 0571613 A	01/12/93
		JP 6506362 T	21/07/94
		US 5766886 A	16/06/98
		US 5770196 A	23/06/98
		US 5821123 A	13/10/98
		US 5869619 A	09/02/99
WO 9516038 A2	15/06/95	AU 699249 B	26/11/98
		AU 1033395 A	27/06/95
		BR 9408278 A	17/12/96
		CA 2178622 A	15/06/95
		CN 1142855 A	12/02/97
		CZ 9601628 A	15/01/97
		EP 0737250 A	16/10/96
		FI 962377 A	30/07/96
		HU 75553 A	28/05/97
		HU 9601574 D	00/00/00
		IL 111926 D	00/00/00
		JP 9509307 T	22/09/97
		LV 11631 A,B	20/12/96
		NO 962346 A	06/08/96
		NZ 276170 A	27/05/98
		PL 314908 A	30/09/96
		SG 48420 A	17/04/98
		SK 73596 A	07/05/97
		US 5861155 A	19/01/99
		ZA 9409341 A	24/05/96
WO 9011091 A1	04/10/90	CA 2049342 A	28/09/90
		EP 0465513 A	15/01/92
		JP 4504253 T	30/07/92
EP 0531539 A1	17/03/93	SE 0531539 T3	
		AU 645187 B	06/01/94
		AU 1337592 A	06/10/92
		CA 2082169 A	09/09/92
		DE 69225757 D,T	03/12/98
		EP 0841067 A	13/05/98
		FI 924982 A	04/11/92
		JP 5065233 A	19/03/93
		WO 9215331 A	17/09/92

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/03/99

International application No.
PCT/SE 99/00049

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8911298 A1	30/11/89	DE 6890533 U	15/04/93
		EP 0417191 A,B	20/03/91
		SE 0417191 T3	
		JP 3504499 T	03/10/91